Effects of Freezing, Thawing, and Cooking on the Appearance of Highbush Blueberries

G.M Sapers, A.M. Burgher, J.G. Phillips, and S.B. Jones
U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, Philadelphia, PA 19118

E.G. Stone
U.S. Department of Agriculture, Agricultural Research Service, Rutgers University Blueberry and Cranberry Research Center, Chatsworth, NJ 08019

Additional index words. *Vaccinium corymbosum*, cultivars, wax, bloom, cuticle, color, anthocyanin, pH

Abstract. **Increases in the darkness and redness of both thawed and cooked highbush blueberries (*Vaccinium corymbosum* L.), indicated by tristimulus measurements, were cultivar-related but not dependent on blueberry pH or anthocyanin content. Waxy bloom was retained in thawed berries but lost during cooking. Pigmented exudate appeared with some cultivars during thawing. Differences among cultivars in exudate formation and reddening during thawing are explained in terms of changes in epidermal cells, cuticle, and wax structure which were observed by light and electron microscopy. The color of blueberry cooking water depended primarily on the berry anthocyanin content, acidity, and the extent of leaching.**

The appearance of fresh and processed small fruits is determined by such factors as surface pigmentation; structural features on the surface imparting glossiness or glaucousness; the presence of pigmented exudate, syrup, or cooking water on the surface; and the occurrence of shriveling, skin splitting, or other defects. Changes in these factors can occur during frozen storage, thawing, and cooking as the result of such processes as enzymatic browning (11), the enzymatic or thermal degradation of pigments (13, 16), anthocyanin complex formation (21), pigment formation from leucoanthocyanins (12), the leakage of pigments intracellularly (10) or into cooking water (4) or syrup (7), the loss of waxy bloom (15), and the physical disruption of fruit tissues. The color of blueberries is well-preserved by freezing (17). Cultivars having a light blue color, which is due to a heavy coating of surface bloom, are preferred for freezing and canning (5).

Previous research in this laboratory on factors affecting the color of raw blueberries demonstrated cultivar differences in the total anthocyanin content, the pattern of individual anthocyanins, berry pH, the tendency of the juice to undergo enzymatic browning, and the structure of the waxy surface (19). These differences, as well as changes in appearance resulting from processing, might be expected to limit the suitability of some cultivars for processing. The objective of the present study was to determine the magnitude and underlying cause of changes in the appearance of highbush blueberry cultivars resulting from freezing, thawing, and cooking.

Materials and Methods

Source and preparation of blueberry samples. Ripe samples of 11 highbush blueberry cultivars ('Berkeley', 'Bluetta', 'Bluecrop', 'Blueray', 'Burlington', 'Collins', 'Coville', 'Earliblue', 'Elliott', 'Jersey', and 'Weymouth') were harvested at the USDA, Rutgers University Blueberry and Cranberry Research Center, Chatsworth, N.J. in 1981 and 1982. Sample ripeness was judged at the time of harvest on the basis of skin color, ease of detachment, and the flavor of representative berries. Raw samples had been evaluated previously for differences in color and composition (19). Within a few hours following harvest, the berries were placed in a 1°C refrigerator for short-term storage. Berries were cleaned, packaged in 2-liter polyethylene freezing containers, and refrigerated or placed in a −13° freezer. The containers were lined up in a single row directly facing the blower to assure uniform and rapid freezing. After several days' equilibration, the containers were transferred to the freezer shelves.

Evaluation of frozen and thawed blueberries. After 2–3 months of storage, berry samples were thawed overnight in a refrigerator and examined by 2 of the authors for differences in bloom. Tristimulus reflectance measurements were performed on the thawed samples (intact berries) with a Gardner XL-23 tristimulus colorimeter (19) after the berries had been "blotted" on a large, absorbent cleaning tissue to remove adhering droplets of condensate or exudate.

Evaluation of cooked blueberries and cooking water. Cooking trials were conducted with refrigerated fresh samples or frozen samples that had been thawed overnight in a refrigerator. About 100 g of berries were added to 50 ml of boiling, distilled water in a 125 × 65 mm evaporating dish, covered with a watch glass, and cooked for 2 min if fresh or for 1 min if frozen and thawed. After cooking, the berries in their cooking water were cooled to about 20°C in a refrigerator, weighed, and additional water added to make up for the evaporation loss. Berries were drained in a 350-ml Buchner funnel with a coarse, fritted disk under suction, and the highly pigmented cooking water was collected.

Tristimulus reflectance measurements were made on the drained, cooked berries after blotting on absorbent tissues. Total anthocyanin in the thawed and/or cooked berries was determined by spectrophotometric analysis at 543 nm of an acidified ethanolic extract with a Perkin-Elmer Model 552 UV-visible spectrophotometer (20).

The pH, titratable acidity, and soluble solids content of the cooking water were measured as described previously (19). Absorbance measurements at the visible absorption maximum (518–524 nm) were made on cooking water samples or aqueous di-

Received for publication 14 June 1983. Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned. The authors acknowledge the technical assistance of Mercedes Fernandez, Sandra P. Graham, Grace Yong, Olena M. Zacharczuk, Beverly Maleeff, and Timothy Dobson. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

lutions thereof, adjusted to the original cooking water pH with 0.5 N HCl. In addition, an estimate of the anthocyanin content of the cooking water was obtained by the pH differential spectrophotometric method of Fuleki and Francis (3), as modified by Sapers et al. (20).

Microscopy. Segments of fresh or thawed blueberries were fixed overnight in 5% glutaraldehyde in 0.08 M sodium cacodylate buffer, pH 7.4, at 4°C, and postfixed in 2% osmium tetroxide in veronal acetate buffer. Tissue was dehydrated in acetone and embedded in Spurr resin. For light microscopy, 2-μm sections of the epoxy-embedded tissue were stained with Epoxy Tissue Stain (Electron Microscopy Sciences). For transmission electron microscopy (TEM), ultra-thin sections (700 Å) were stained with uranyl acetate and lead citrate. For scanning electron microscopy (SEM), the procedure used by Albrigo et al. (1) essentially was followed. Strips of epidermis, with a minimal amount of underlying tissue adhering, were cut from fresh or thawed berries with a razor blade and laid flat in a desiccator for 48 hr. The dry tissue then was attached to specimen mounts with silver paint and coated with 15 nm of gold-palladium. Specimens were observed with a Zeiss EM 10 transmission electron microscope, operated at 50 kV, or with a JEOL 50A scanning electron microscope, operated at 15 kV.

Statistical analyses. Statistical analyses were performed with the Statistical Analysis System (SAS Institute, Inc., Cary, N.C.), General Linear Models Procedure. Analysis of variance was used to test the effects of treatments, cultivars, and sampling. Comparisons of means were made by Duncan's multiple range test or the Bonferroni least significant difference method (14).

Results and Discussion

Berry appearance. Thawing and/or cooking blueberries resulted in darkening of fruit (decreasing tristimulus L values) for most cultivars in comparison to raw, unfrozen controls (Table 1). Cooking resulted in more darkening than did freezing and thawing. Waxy bloom was at least partially retained during freezing and thawing but was lost during cooking in all samples. We demonstrated a correlation between bloom and the L-value with raw blueberries (19). This relationship also may apply to thawed and cooked samples. 'Elliott' berries differed from the other cultivars because of their tendency to leak exudate when thawed, resulting in a visibly wet surface. Decreases in L in thawed and/or cooked blueberries were accompanied by increases in a_L (more red) and b_L (less blue). The hue angle θ (defined as $\tan^{-1} b_L/a_L$) also increased, indicating a shift in berry color from blue ($\theta = 270°$) towards a more red hue ($\theta = 0°$ or 360°). Tristimulus data for berries that were cooked fresh or after freezing and thawing were similar. 'Burlington' showed the least change in hue when thawed of the 11 cultivars examined, while 'Elliott' underwent the greatest change. 'Burlington' also exhibited the smallest increase in hue with cooking while 'Bluecrop' and 'Weymouth' (data not shown) underwent the largest increase; the other cultivars, including 'Bluetta', 'Blueray', 'Collins', and 'Earliblue' (data not shown), exhibited an intermediate increase in hue with cooking.

Color changes in frozen blackberries have been attributed to intracellular pH changes (10). The leakage and intermingling of anthocyanins, acids, and other cell constituents, resulting from freezing, thawing, and cooking, also may explain our results.

Table 1. Effects of freezing, thawing, and cooking on tristimulus reflectance parameters, bloom, and total anthocyanin content of highbush blueberry cultivars.[z]

Cultivar	pH	Treatment	Bloom	Reflectance parameters				Total anthocyanin[x]
				L	a_L	b_L	θ[y]	
Berkeley	3.5	Raw, unfrozen	Extensive	23.2 a	1.5 a	−5.5 b	286 c	148 a
		Frozen, thawed	Retained	18.2 b	2.5 a	−4.6 b	298 bc	---
		Cooked	Lost	14.9 b	2.9 a	−2.5 a	319 ab	154 a
		Frozen, thawed, cooked	Lost	11.2 c	3.6 a	−2.5 a	326 a	146 a
Bluecrop	3.4	Raw, unfrozen	Extensive	21.8 a	1.4 b	−5.3 b	286 b	84 a
		Frozen, thawed	Retained	17.8 ab	3.5 ab	−4.3 ab	311 ab	---
		Cooked	Lost	14.6 b	5.6 a	−3.5 ab	328 a	68 a
		Frozen, thawed, cooked	Lost	13.8 b	5.4 a	−3.0 a	330 a	64 a
Burlington	3.3	Raw, unfrozen	Extensive	20.6 a	1.4 b	−4.6 b	287 b	269 a
		Frozen, thawed	Retained	16.7 ab	1.6 b	−4.1 b	291 b	---
		Cooked	Lost	14.8 b	1.6 b	−1.9 a	311 a	246 a
		Frozen, thawed, cooked	Lost	12.0 b	2.2 a	−2.3 a	319 a	267 a
Coville	3.0	Raw, unfrozen	Moderate	18.2 a	1.5 b	−4.0 a	291 b	146 a
		Frozen, thawed	Retained	16.4 ab	4.0 ab	−4.4 a	312 a	---
		Cooked	Lost	13.9 ab	4.3 a	−2.6 a	329 a	132 a
		Frozen, thawed, cooked	Lost	12.0 b	4.7 a	−3.3 a	325 a	145 a
Elliott	2.9	Raw, unfrozen	Extensive	21.6 a	1.2 b	−4.6 b	284 b	224 a
		Frozen, thawed	Retained	21.4 a	3.9 a	−2.8 ab	324 a	---
		Cooked	Lost	13.0 b	3.1 a	−2.5 a	321 a	266 a
		Frozen, thawed, cooked	Lost	11.0 b	3.9 a	−2.4 a	329 a	222 a
Jersey	3.5	Raw, unfrozen	Moderate	19.1 a	1.6 b	−4.2 a	291 b	142 a
		Frozen, thawed	Retained	15.0 a	3.7 a	−4.1 a	312 a	---
		Cooked	Lost	13.5 a	3.2 ab	−2.7 a	319 a	132 ab
		Frozen, thawed, cooked	Lost	10.4 a	3.2 ab	−2.6 a	321 a	81 b

[z]Means of duplicate analyses performed on duplicate samples. Mean separation in columns for individual varieties by Bonferroni least significant difference method, 5% level.
[y]Hue angle $\theta = \tan^{-1} b_L/a_L$.
[x]Absorbance of acidified ethanolic extract at 543 nm × dilution factor.

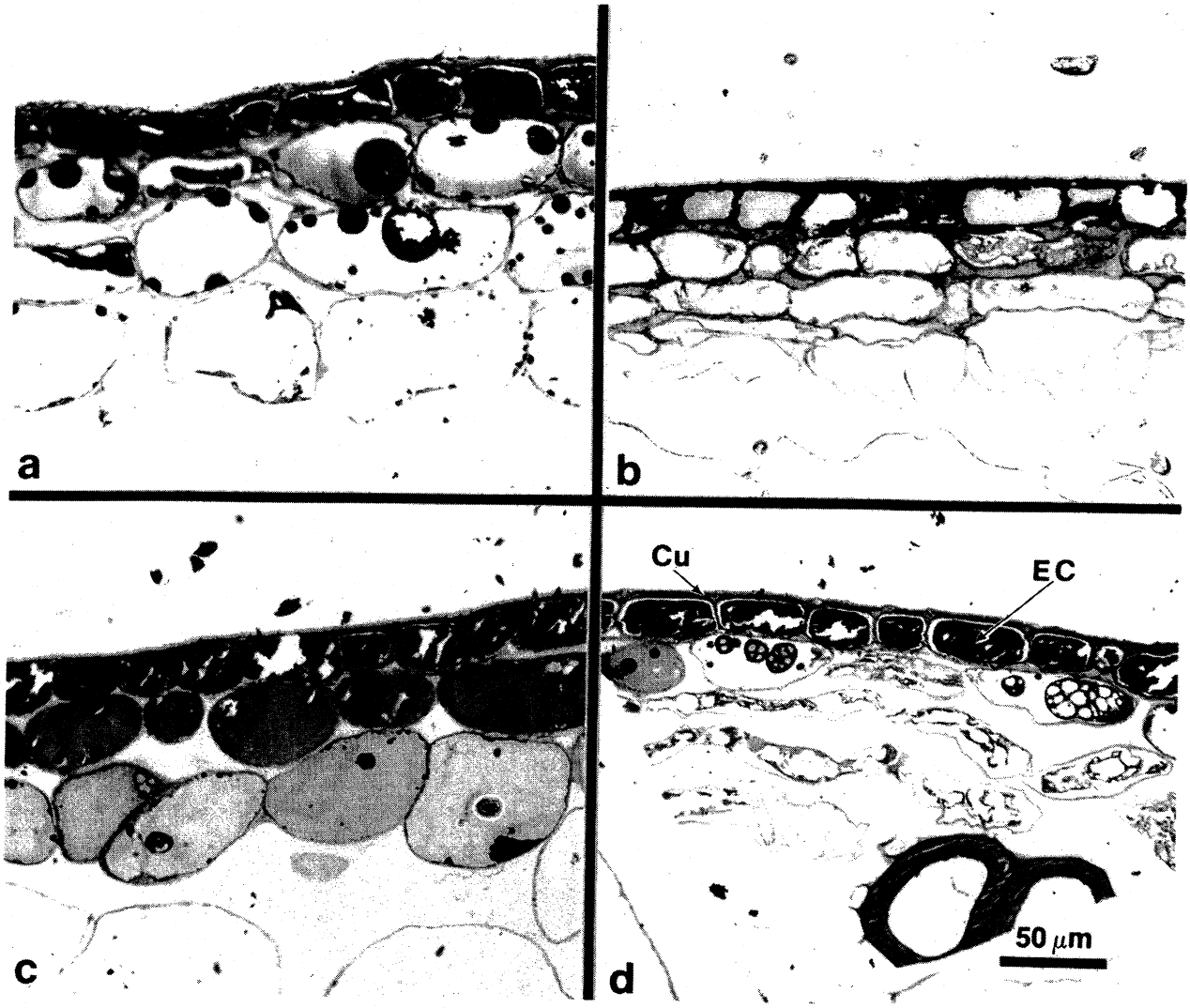

Fig. 1. 'Elliott' and 'Burlington' ripe berries, fresh vs. thawed. Cross sections through cuticle (Cu), epidermal cells (EC), and subepidermal cells. Light micrographs (300×): **a)** 'Elliott', fresh; **b)** 'Elliott', thawed; **c)** 'Burlington', fresh; and **d)** 'Burlington', thawed.

Our data do not show a relationship between the tristimulus values and the sample pH (i.e., 'Coville' and 'Elliott' vs. 'Bluecrop' and 'Jersey'). However, while pH differences between blueberry samples may appear large in terms of anthocyanin color expression, they may be relatively small when compared to pH changes at the cellular level resulting from leakage. Our data also show no relationship between the tristimulus parameters and the total anthocyanin content of raw or processed blueberries, probably because of the high pigment content of the epidermal cells.

Microscopy observations. Microscopic observations of berry samples were carried out to explain differences in fruit appearance and reflectance properties. Light microscopy revealed the loss of pigment from epidermal and subepidermal cells of thawed berries. Some variation among cultivars was evident, illustrated particularly by 'Elliott' and 'Burlington' (Fig. 1). Fresh 'Elliott' berries (Fig. 1a), like all the cultivars examined by microscopy, contained pigment in at least 3 outer layers of cells. As a consequence of freezing and thawing (Fig. 1b), 'Elliott' lost nearly all observable pigment from these layers. In contrast, 'Burlington' (Fig. 1c and 1d) retained its epidermal cell pigment during freezing and thawing.

Since 'Elliott' was observed to lose juice and pigment directly through the skin after thawing, we examined the ultrastructure of the cuticular region and the morphology of the surface wax. TEM revealed no obvious differences in cuticle structure between 'Elliott' and 'Burlington', either before or after freezing and thawing (Fig. 2). Surface wax is often removed by solvents during sample preparation for TEM, and its presence or absence in Fig. 2 is not significant. It appeared that the epidermal cell wall component of 'Elliott' skin was less densely stained after thawing (Fig. 2b). This remains to be confirmed, however, since variations in section thickness were found to influence contrast markedly in the cuticle layers.

SEM images of surface wax of 'Elliott' and 'Burlington', before and after freezing, are shown in Fig. 3. The wax of unfrozen 'Elliott' consisted of discrete plates (Fig. 3a), while unfrozen 'Burlington' had a more uniform and continuous wax coating (Fig. 3d). Freezing and thawing had little or no effect on 'Burlington' wax (Fig. 3e), but caused the surface wax of 'Elliott' to appear softened or partially annealed (Fig. 3b). In several locations on thawed 'Elliott', the surface had no visible wax (Fig. 3c).

The apparent annealing of wax structures after freeze-thaw was observed in varying degrees for each of the 4 cultivars tested, and for berries of different maturities. Such alterations may reflect "weathering" (2, 18), caused by volume changes in the wax components with changing temperature, and may be de-

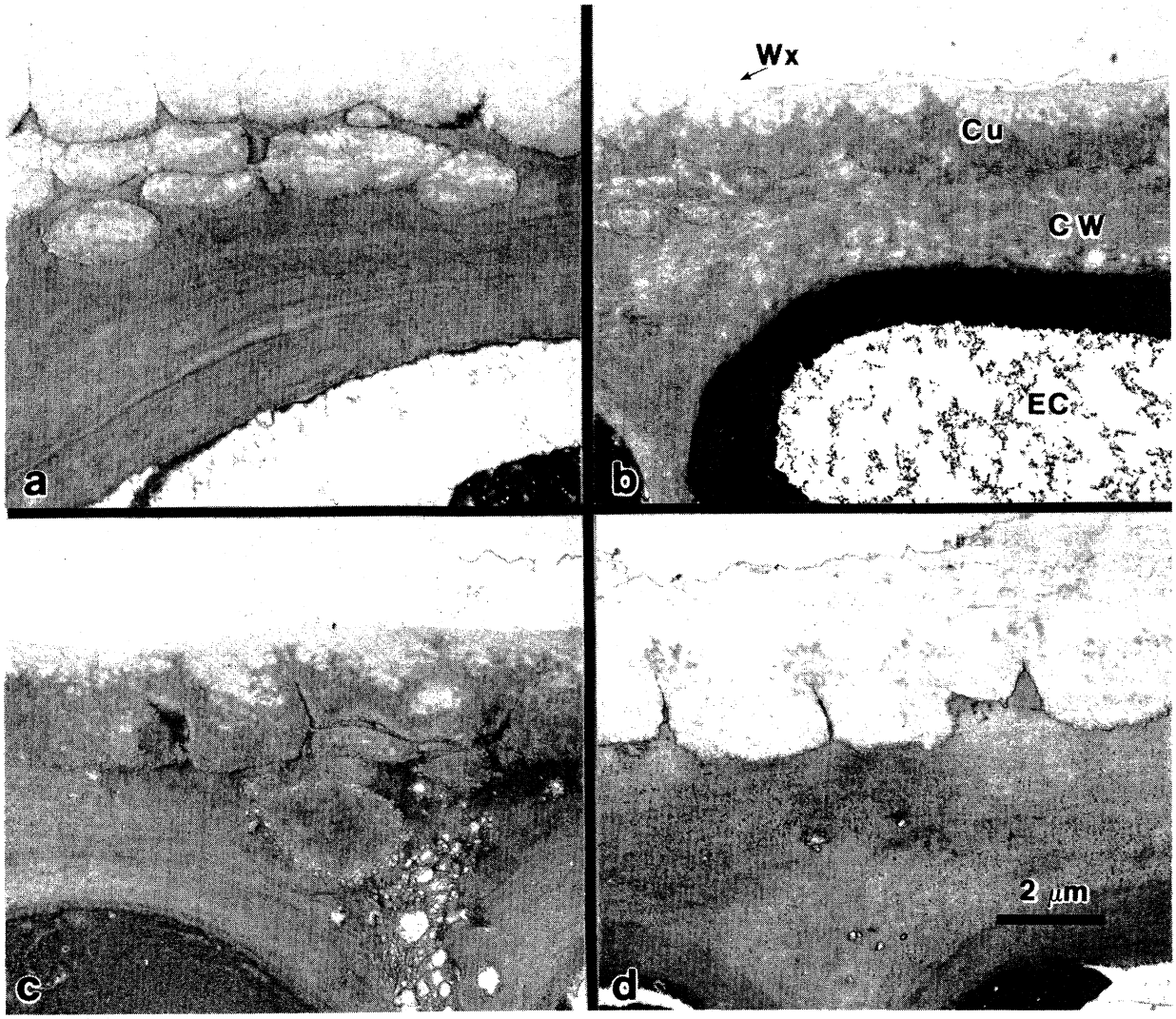

Fig. 2. Cross sections through cuticles of 'Elliott' and 'Burlington', fresh and thawed. Cu = cuticle; CW = cell wall; EC = epidermal cell; and Wx = wax. Transmission electron micrographs (8000×): **a)** 'Elliott', fresh; **b)** 'Elliott', thawed; **c)** 'Burlington', fresh; and **d)** 'Burlington', thawed.

pendent on the particular wax composition of a given cultivar (1, 9). Phase changes in the epicuticular wax at low temperatures are not likely (10). The upright wax forms that contribute to the appearance of bloom (1) were not affected by freeze-thaw.

The SEM and TEM observations suggest that the wax coating of 'Elliott' blueberries may be a less effective barrier to diffusion than 'Burlington' wax, explaining the apparent ease with which pigment-bearing cellular fluids leak through the skin of thawing 'Elliott' berries. Albrigo et al. (1) demonstrated that water loss from the fruit of native *Vaccinium elliotti* Chapm. (a wild blueberry) was a function of the cuticular wax content and composition.

Composition and spectral properties of cooking water. The water in which blueberries are cooked contributes significantly to the overall appearance of the cooked product (normally not separated from the cooking liquid) because of its intense color, developed from the anthocyanins and acids extracted from the berries. The extent of extraction varied significantly among the 11 cultivars under standardized cooking conditions. The soluble solids content of individual cooking water samples ranged between 1.2–1.3% for 'Berkeley', 'Bluecrop', and 'Burlington', and between 3.3–3.5% for 'Earliblue', 'Weymouth', and 'Bluetta' (Table 2). The anthocyanin concentration in cooking water samples varied over a 10-fold range, roughly paralleling the soluble solids content ($r = 0.9$), as would be expected with a leaching loss. Differences among cultivars in the extent of leaching during cooking probably were due to differences in epidermal cell wall and/or cuticle permeability and berry size (surface area/volume). Factors controlling the extent of leaching during cooking as well as exudate formation during thawing are currently under investigation.

Color expression by the leached anthocyanins, as indicated by absorbance values at the visible absorption maximum, was governed both by the pigment concentration and the pH. The pH of cooking water samples ranged between 3.0 ('Elliott') and 3.6 ('Burlington' and 'Bluecrop'), corresponding to differences in cooking water titratable acidity between 0.05–0.06% for the higher pH samples and 0.34% for 'Elliott'. This is a consequence of the higher acidity of the 'Elliott' berries (1.3% citric acid, as compared to 0.6–0.7% for 'Burlington' and 'Bluecrop'). Such acidity differences may be indicative of differences in sample maturity at the time of harvest. However, repeated samplings of apparently ripe fruit from 'Elliott', taken over 2 seasons, consistently were high in acidity (19), suggesting that the high acidity of this cultivar has a genetic basis (6). Because of its greater acidity, 'Elliott' cooking water was colored more intensely than that from 'Bluetta' or 'Weymouth' samples at comparable anthocyanin concentrations.

Table 2. Composition and spectral properties of cooking water from fresh highbush blueberries.[z]

Cultivar	pH	Titratable acidity (% citric)	Soluble solids (%) at 20°C	Anthocyanin concn.[y]	$A_{\lambda max}$ × diln factor[x]
Berkeley	3.5 ab	0.04 d	1.3 cd	350 c	0.74 d
Bluetta	3.4 ab	0.16 b	3.3 a	2160 ab	5.29 bc
Bluecrop	3.6 a	0.05 d	1.2 d	440 c	0.80 d
Blueray	3.4 ab	0.11 bcd	2.0 bc	960 abc	2.65 cd
Burlington	3.6 ab	0.06 cd	1.3 cd	930 abc	2.00 d
Collins	3.5 ab	0.10 bcd	2.6 ab	1610 abc	2.08 d
Coville	3.3 b	0.11 bcd	1.9 bcd	960 abc	2.54 cd
Earliblue	3.5 ab	0.12 bc	3.1 a	1320 abc	3.30 cd
Elliott	3.0 c	0.34 a	2.2 b	2240 a	9.97 a
Jersey	3.5 ab	0.07 cd	2.3 b	870 bc	1.60 d
Weymouth	3.3 b	0.15 b	3.2 a	2120 ab	7.14 b

[z]Means of duplicate analyses performed on each of 2 harvests. Mean separation in columns by Duncan's multiple range test, 5% level.
[y]pH differential method for aqueous solutions: total anthocyanin = $(A_{\lambda max\ pH\ 1.0} - A_{\lambda max\ pH\ 4.5}) \times$ dilution factor.
[x]At cooking water pH.

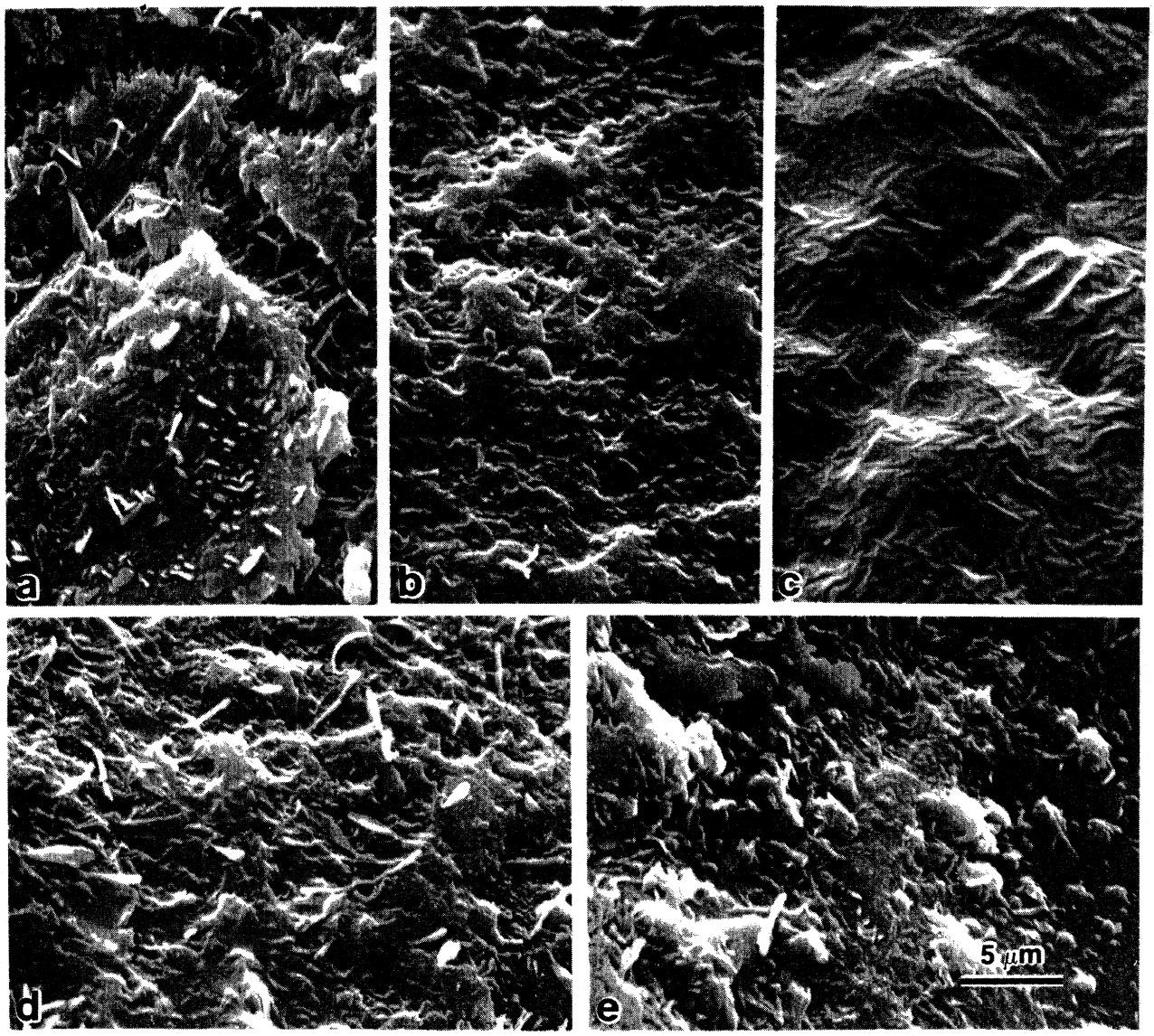

Fig. 3. 'Elliott' and 'Burlington' ripe berries with bloom, fresh vs. thawed. SEM images of wax surface (3000 ×): **a)** 'Elliott', fresh; **b–c)** 'Elliott', thawed; **d)** 'Burlington', fresh; and **e)** 'Burlington', thawed.

Conclusions

Changes in the appearance of highbush blueberries during freezing, thawing, and cooking result from the loss of waxy bloom, leakage of cellular fluids through the skin, and an increase in the redness of skin anthocyanins.

The color of the cooking water, which influences cooked berry appearance, is determined by the concentrations of extracted anthocyanins and acids which are related to berry anthocyanin and acid contents as well as to skin permeability.

Differences among blueberry cultivars in the appearance of processed berries or cooking water can be attributed to cultivar or maturity differences in the factors described above.

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